Influence of brain catecholamines on the development of fatigue in exercising rats in the heat

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The purpose of the present study was to identify the effects of an acute injection of a dual dopamine (DA)/noradrenaline (NA) reuptake inhibitor (bupropion) on exercise performance, thermoregulation and neurotransmitters in the preoptic area and anterior hypothalamus (PO/AH) of the rat during exercise in the heat. Body core temperature (T_{core}), brain temperature (T_{brain}) and tail skin temperature (T_{tail}) were measured. A microdialysis probe was inserted in the PO/AH, and samples for measurement of extracellular DA, NA and serotonin (5-HT) levels were collected. Rats received either bupropion (17 mg kg^{-1}; hot-BUP) or saline (1 ml kg^{-1}; hot) 20 min before the start of exercise and ran at a speed of 26 m min^{-1} until exhaustion in a warm environment (30°C). Rats also ran until exhaustion in a cool environment (18°C; cool). Running time to exhaustion was significantly influenced by the ambient temperature, and it was increased by bupropion in the heat (cool, 143.6 ± 21 min; hot, 65.8 ± 13 min; hot-BUP, 86.3 ± 7.2 min). T_{core} and T_{brain} at exhaustion were significantly higher in the bupropion group compared to the cool and hot groups, respectively. T_{tail} measured at exhaustion was not significantly different between the two hot conditions. Extracellular concentrations of DA and NA in the PO/AH increased during exercise, and was significantly higher in the bupropion than in cool and hot groups ($P < 0.05$). No differences were observed between groups for 5-HT levels. These results suggest that DA and NA in the PO/AH might be responsible for the increase in exercise performance and T_{core} and T_{brain} in the bupropion group in hyperthermia. Moreover, these results support previous findings in humans that acute bupropion ingestion increases T_{core} during exercise in the heat, indicating the possibility of an important role for DA and NA in thermoregulation.

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Many major sport events are held in extremely hot conditions, and recent summer Olympic Games are no exception; these have taken place under high ambient temperature such as in Atlanta, GA, USA in 1996 and Athens, Greece in 2004. This trend is likely to continue as athletes begin to prepare for what will probably be another hot and humid Olympic Games in 2008 in Beijing, China (Quod *et al.* 2006).

It is well accepted that the human body is able to thermoregulate efficiently during exercise in a range of cool to moderate ambient conditions. However, this has been shown to be more difficult during exercise in hot conditions. It is established that exercise performance is impaired at high ambient temperature (Galloway & Maughan, 1997; Hargreaves & Febbraio, 1998; Parkin *et al.* 1999). It is interesting to note that some studies indicate that exhaustion during prolonged exercise in the heat appears to coincide with the attainment of a critical internal body temperature of around 40°C (Nielsen *et al.* 1993; Gonzalez-Alonso *et al.* 1999). The attainment of this so-called critically high body core temperature (T_{core}) during prolonged exercise has been suggested to result in a loss of central nervous system (CNS) drive (Nielsen *et al.* 2001), and has been associated with increased perception of effort (Nybo & Nielsen, 2001), and altered electroencephalographic brain activity of the frontal cortex (Nielsen *et al.* 2001). As a result, the attainment of a critically high T_{core} and brain temperature (T_{brain})

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has been proposed as an important factor in limiting endurance performance in the heat in both human (Nielsen *et al.* 1993; Cheung & McLellan, 1998; Gonzalez-Alonso *et al.* 1999) and animal studies (Fuller *et al.* 1998; Walters *et al.* 2000).

Brain catecholamines are known to play a role in arousal, mood, motivation, vigilance, anxiety and reward mechanisms and therefore could, if adversely affected, impair exercise performance (Davis & Bailey, 1997). The depletion of central catecholamine levels has been linked to CNS fatigue by a number of research groups (Chaouloff, 1989; Davis, 2000). A series of animal studies conducted by Davis & Bailey (1997) demonstrated that brain serotonin (5-HT) and dopamine (DA) activity were elevated during exercise, but a marked fall in tissue DA content was apparent at the point of exhaustion. This observation resulted in the suggestion that the ratio of 5-HT to DA activity may be important for the development of central fatigue. Different catecholaminergic reuptake inhibitors have been used in humans in order to evaluate the effects of an increased neurotransmission on exercise performance and on the hormonal response to exercise (Meeusen *et al.* 1997, 2001; Piacentini *et al.* 2002*a*,*b*, 2004). Brain catecholamines are also considered to be involved in thermoregulation especially in the preoptic area and the anterior hypothalamus (PO/AH), which are the primary loci for maintenance of body temperature (Boulant & Dean, 1986). A number of studies have investigated the relationship between DA, noradrenaline (NA) and thermoregulation in the PO/AH (Myers & Yaksh, 1968; Cox & Lee, 1980; Clark & Lipton, 1986; Quan *et al.* 1991, 1992; Hasegawa *et al.* 2000).

Recently we found that acute ingestion of the dual DA/NA reuptake inhibitor bupropion improved time trial exercise performance of cyclists only in a warm environment (Watson *et al.* 2005). The rectal temperature of the subjects during intensive exercise was significantly higher in the bupropion group compared to the placebo group, almost reaching critical limits (40°C). It is noteworthy that this response appeared to occur without any change in the subjects' perceived exertion or thermal sensation, and may potentially increase the risk of developing heat stroke and heat illness. These results suggest that during exercise in the heat, bupropion may override the inhibitory signals arising from the CNS that cause exercise to stop when close to the critical temperature. The English cyclist Tom Simpson collapsed and died from heat illness in 1967 at the Tour de France during intensive exercise in the heat after taking amphetamines. This may be explained by the results of the study evaluating the effects of bupropion on performance in the heat, because amphetamines are thought to act directly on catecholaminergic neurones to produce a marked elevation in extracellular DA concentrations.

Moreover, an acute injection of bupropion in freely moving rats induced an increase in T_{brain} and T_{core} with a decreased heat loss response (Hasegawa *et al.* 2005). By using microdialysis, we registered an increase in DA and NA levels in the PO/AH after bupropion injection. An acute injection of bupropion has been shown to increase DA and NA levels in the hippocampus (Piacentini *et al.* 2003) and in the PO/AH (Hasegawa *et al.* 2005), but only in the latter study was the effect on temperature investigated, in accordance with previous studies on humans (Watson *et al.* 2005) where bupropion increased exercise performance in a warm environment.

From these previous studies, it is known that bupropion improves exercise performance in humans in a warm environment, and that thermoregulation in rat in normothermia and at rest is disturbed. However, at this stage we do not know whether exhaustion or central fatigue is associated with decreased catecholaminergic neural activity and whether an increased catecholaminergic neural activity might 'override' the inhibitory effect of hyperthermia. Therefore, the purpose of the present study was to verify, by means of brain microdialysis, the effects of an acute injection of bupropion on thermoregulation and exercise performance in running rats in a warm environment.

Methods

Animal treatment

Male Wistar rats (Charles River Laboratories, Iffa-Credo, Lyon, France), weighing 300–350 g, were used in all experiments. Animals were housed in a room at normal ambient temperature, on a 12 h light–dark cycle (lights on at 08.00 h). Animals had a standard diet with free access to food and water throughout the experiments. Animal experiments were carried out according to the National/European guidelines on animal experimentation and were approved by the Ethical Committee for Animal Experiments of the Faculty of Medicine and Pharmacy of Vrije Universiteit Brussel. All efforts were made to minimize animal suffering and the minimum number of animals necessary to produce reliable scientific data was used.

Exercise familiarization sessions and surgery

Rats were trained for 1.5 weeks on a treadmill using mild electric shocks. After a few running sessions, animals were capable of running without electric shock. Exercise intensity and duration were gradually increased up to a speed of 26 m min^{-1} and duration of 80 min. We initially trained 50 rats in this study. After the training protocol was finalized, 43 rats were able to run smoothly, without any aversive stimulus during running. These

rats were anaesthetized with pentobarbital (60 mg kg^{-1}, I.P.). A telemetry device (TA10TA-F20, Data Sciences International, MN, USA) was implanted in the peritoneal cavity (Hasegawa *et al.* 2000). After a 6 day recovery period, rats were anaesthetized with pentobarbital (60 mg kg^{-1}, I.P.), and placed on a stereotaxic frame. The skull was exposed and two intracerebral guides (MAB 6.14.IC, Microbiotech, Stockholm, Sweden) were implanted. One guide was inserted in the left PO/AH (anterior, −0.3 mm; lateral, −0.8 mm; ventral, +6.7 mm; relative to bregma) for the microdialysis probe, and one in the right frontal cortex (anterior, +2.5 mm; lateral, +3.2 mm; ventral, +2.0 mm; relative to bregma) for the thermocouple probe to measure T_{brain} (Paxinos & Watson, 1986; Hasegawa *et al.* 2005). The cannulae were secured to the skull using dental cement (Durelon, Germany). Postoperative analgesia was provided by giving a single injection of ketofen (4 mg kg^{-1}, I.P.) to each rat. This was followed by a treadmill re-adaptation period for 1 week before microdialysis experiments were carried out.

Experimental procedures

On the day of the experiment, rats were anaesthetized with sevoflurane 4% and oxygen insufflated into a transparent chamber. After induction, the rat was maintained under anaesthesia to change the probes, using 1.5% sevoflurane delivered with oxygen at 0.8 l min^{-1} via a facemask (Van Hemelrijck *et al.* 2003; Hasegawa *et al.* 2005). The guides were replaced by a microdialysis probe with a membrane length of 2 mm (MAB 6.14.2, Microbiotech) and a thermocouple probe in the prefrontal cortex (HYP-O-SLE, Omega Corporation, Stamford, CT, USA). The thermocouple for skin temperature measurement was also attached with tape on the dorsal surface of the skin about 10 mm from the base of the tail. The microdialysis probes were connected to a microinfusion pump (CMA 100, CMA Microdialysis, Stockholm, Sweden) and were perfused with Ringer solution (147 mM NaCl, 4 mM KCl and 2.3 mM $CaCl_2$) at a flow rate of 1 μl min^{-1}. Microdialysis sampling was started 2 h after probe implantation. Then, after 2 h of baseline collections, rats randomly received either an intraperitoneal injection of 17 mg kg^{-1} of bupropion hydrochloride (BUP), dissolved in physiological saline (GlaxoSmithKline, Hertfordshire, UK), or saline (1 ml kg^{-1}) 20 min before exercise. Rats exercised at a speed of 26 m min^{-1} on a treadmill until exhaustion in a cool (18°C, cool, $n = 8$) or warm environment (30°C; hot, $n = 8$; hot-BUP, $n = 9$). Exhaustion was considered to have occurred when the rat was unable to keep pace with the treadmill and lay flat on, and stayed on the grid positioned at the back of the treadmill for a period of 30 s despite gently being pushed with sticks or breathed on. We confirmed that the exhausted rats were unable to move for at least 5 min on the treadmill.

Sampling

During the experiments, microdialysis samples (20 μl) were collected every 20 min prior to and during exercise. The samples were protected from oxidation by addition of 5 μl antioxidant solution containing (mM): L-cysteine 3.3, Na_2EDTA 0.27, acetic acid 100 mM and ascorbic acid 0.0125. T_{brain}, T_{core} and tail skin temperature (T_{tail}), an index of heat loss response, were measured every 20 min.

Chromatographic assays for the determination of DA, NA and 5-HT levels in dialysates from PO/AH

For the analysis of DA, NA and 5-HT levels, an off-line microbore liquid chromatography (LC) assay was used with automatic injection (10 μl) of the samples, as previously described in detail (Sarre *et al.* 1997; Clinckers *et al.* 2004). To summarise, the assay was based on ion-pair, reversed-phase chromatography (C8, 5 μm; 100 × 1 mm), coupled to single-channel electrochemical detection (Decade, Leiden, the Netherlands). The mobile phase consisted of 27 ml acetonitrile and 200 ml aqueous buffer containing (mM): sodium acetate trihydrate 0.1, citric acid monohydrate 20, decane sulphonic acid 2 and sodium EDTA 0.5; adjusted to pH 5.5. The flow rate through the column was 90 μl min^{-1}. Because of the high pH 5.5 of the mobile phase, a low oxidation potential was set (450 mV *versus* Ag–AgCl). The retention times for NA, DA and 5-HT were 3, 6 and 12 min, respectively, with a quantification limit for all compounds between 30 and 60 pM.

Histological examination

At the end of each experiment, rats were killed with an overdose of pentobarbital and the brain was removed. The position of the microdialysis probe was verified in coronal sections (100 μm thick) stained with Chinese ink according to the coordinates described by Paxinos & Watson (1986).

Data collection and statistical analysis

The average concentration of three microdialysis samples for 1 h before drug administration was considered as the baseline and was defined as 100%. Temperature values and microdialysis samples relative to the baseline value were expressed as mean ± S.D. Differences between data were evaluated for statistical significance by using two-factor (time and conditions) repeated-measures ANOVA followed by Scheffe's *post hoc* tests. $P < 0.05$ was regarded as statistically significant.

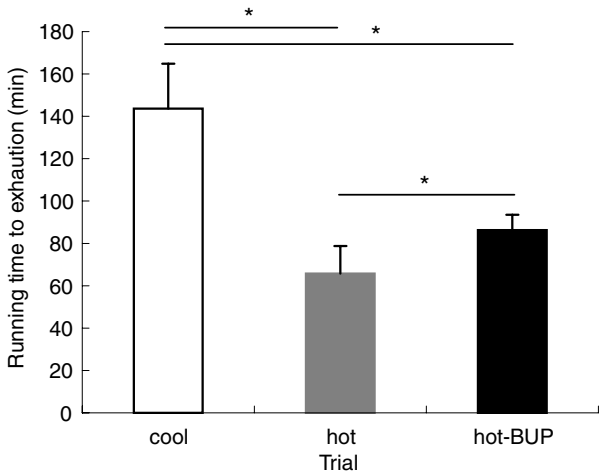

Figure 1. Running time to exhaustion
*Significant difference between conditions ($P < 0.05$). Values are mean ± S.D.

Results

Running time to exhaustion was significantly influenced by the ambient temperature with values significantly shorter in the hot and bupropion than the cool group (cool, 143.6 ± 21 min; hot, 65.8 ± 13 min; hot-BUP, 86.3 ± 7.2 min; $P < 0.001$, Fig. 1). Exercise performance increased significantly in the bupropion group compared to the hot group. In fact, rats exercised for longer (hot, 65.8 ± 13 min; hot-BUP, 86.3 ± 7.2 min; $P = 0.02$).

Figure 2 shows the mean changes in T_{core} (Fig. 2*A*) and T_{brain} (Fig. 2*B*) prior to and during treadmill exercise. Bupropion significantly increased T_{core} before the start of exercise (cool, 37.8 ± 0.8°C; hot, 37.6 ± 0.8°C; hot-BUP, 38.5 ± 0.7°C; $P < 0.05$). Treadmill exercise in the heat produced a progressive increase in T_{core} in both hot and bupropion groups; however, T_{core} was significantly higher in the bupropion group at the point of exhaustion (cool, 39.6 ± 0.6°C; hot, 41.0 ± 0.7°C; hot-BUP, 42.3 ± 0.5°C; $P < 0.001$, Fig. 2*A*). Although T_{brain} before treadmill exercise was influenced by the acute injection of bupropion, this difference was not statistically significant ($P = 0.19$). Treadmill exercise linearly increased T_{brain} in both hot and bupropion groups in warm conditions. At the point of exhaustion, T_{brain} was significantly higher in the bupropion group (cool, 39.1 ± 0.8°C; hot, 40.5 ± 0.7°C; hot-BUP, 41.6 ± 0.7°C; $P < 0.05$, Fig. 2*B*).

T_{tail} increased after 10 min of treadmill exercise in the three groups, indicating that vasodilatation had occurred

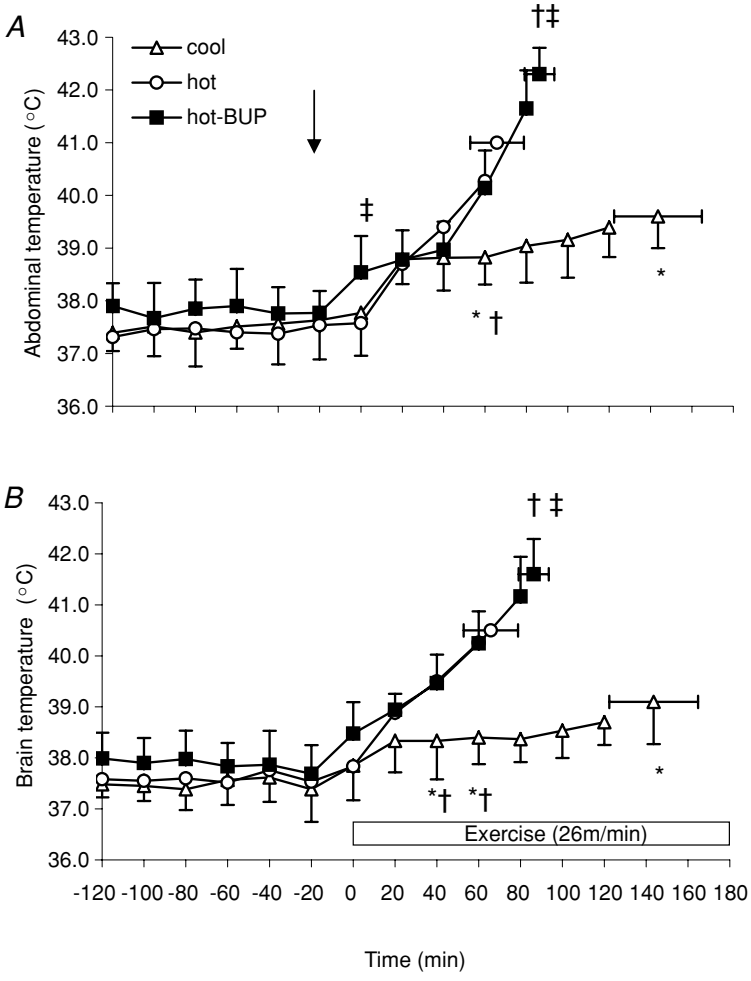

Figure 2. Changes in core temperature (*A*) and brain temperature (*B*)
*Significant difference, cool *versus* hot ($P < 0.05$); †significant difference, cool *versus* hot-BUP ($P < 0.05$); ‡significant difference, hot *versus* hot-BUP ($P < 0.05$). Values are mean ± S.D. Arrow indicates injection of bupropion or saline.

and showing activation of heat loss responses. Thereafter, T_{tail} reached a plateau after 40 min of exercise in the cool group. T_{tail} in the hot and bupropion groups continued to increase slightly throughout the period of exercise. Acute injection of bupropion did not influence T_{tail} during exercise in the heat, although there was a tendency for T_{tail} of the bupropion group to be lower when compared to values recorded during the corresponding period in the hot group (time 20 min: hot, $33.1 \pm 1.6^{\circ}$C; hot-BUP, $30.8 \pm 2.6^{\circ}$C; $P = 0.10$). The final values of T_{tail} were not significantly different between the groups in the heat (hot, $35.4 \pm 1.5^{\circ}$C; hot-BUP, $35.8 \pm 1.3^{\circ}$C; Fig. 3).

Figure 4 shows the mean changes in extracellular DA (Fig. 4*A*), NA (Fig. 4*B*) and 5-HT (Fig. 4*C*) levels in the PO/AH prior to and during exercise. Extracellular DA levels in the PO/AH had significantly increased already by 20 min after bupropion injection (time 0) compared with the cool and hot groups. Extracellular concentrations of DA in the PO/AH increased during exercise, and was significantly higher in the bupropion than in the cool and hot groups reaching peak values after 80 min of exercise (cool, $178 \pm 38\%$; hot, $259 \pm 78\%$; hot-BUP, $534 \pm 206\%$; $P < 0.05$, Fig. 4*A*). Extracellular NA levels also significantly increased 20 min after bupropion injection. During exercise, extracellular NA levels in the PO/AH in the bupropion group was significantly higher than in the cool and hot groups, reaching peak values after 80 min of exercise (cool, $226 \pm 221\%$; hot, $223 \pm 120\%$; hot-BUP, $495 \pm 221\%$; $P < 0.05$, Fig. 4*B*). The level of 5-HT showed no significant change after bupropion injection and treadmill exercise in each condition (Fig. 4*C*). Therefore, no differences were observed between groups for 5-HT.

Statistical analysis of final T_{core} and neurotransmitter levels at exhaustion showed a good correlation only with changes in DA ($R^2 = 0.62$) and NA ($R^2 = 0.58$) but not with 5-HT ($R^2 = 0.31$). The results of T_{brain} and neurotransmitter levels are similar to T_{core} (DA, $R^2 = 0.53$; NA, $R^2 = 0.55$; 5-HT, $R^2 = 0.17$).

Discussion

The main finding of the present study in rats was that an acute injection of the DA and NA reuptake inhibitor bupropion improved exercise performance and induced an increase in both T_{core} and T_{brain} during exercise in a warm environment. These changes in temperature were accompanied by an increase in the extracellular concentrations of DA and NA in the PO/AH in exercising rats measured via *in vivo* brain microdialysis. The present results not only confirm previous results in humans that acute bupropion ingestion enhanced exercise performance with an increased internal body temperature in the heat, but also showed that the acute injection of bupropion acts on the brain by influencing T_{brain} and specific neurotransmitters in the thermoregulatory centre during exercise in the heat. The results also indicate that the so-called critical T_{core} can be bypassed when animals are running in the heat in the presence of high concentrations of catecholamines.

During prolonged exercise in the heat, elevated internal body temperature and increased heat storage have been considered to be limiting factors (Nielsen *et al.* 1993; Fuller *et al.* 1998; Gonzalez-Alonso *et al.* 1999) that reduce CNS drive for exercise performance and precipitate feelings of fatigue (Nielsen *et al.* 1990; Walters *et al.* 2000), thus protecting the brain from thermal damage. Exhaustion appears to coincide with the attainment of an internal body temperature around 40°C. In the present study, T_{core} measured at exhaustion reaching critical values in the hot group, indicating that elevated T_{core} might be one of the major factors limiting endurance performance in the heat and critical temperature during exercise existed in the present study. However, T_{core} at exhaustion was significantly higher in the bupropion group than in the hot group (hot-BUP, $42.3 \pm 0.5^{\circ}$C; hot, $41.0 \pm 0.7^{\circ}$C). Similar results were also seen for T_{brain}. Bupropion administration allowed performance to be maintained despite high rates of heat production resulting in longer time to exhaustion

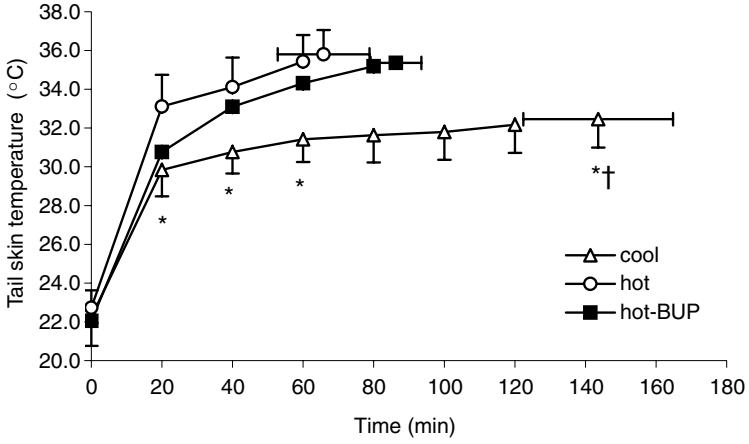

Figure 3. Changes in tail skin temperature during exercise
*Significant difference, cool *versus* hot ($P < 0.05$); †significant difference, cool *versus* hot-BUP ($P < 0.05$); Values are mean ± S.D.

compared to both the cool and hot groups (Fig. 1). This might indicate that manipulation of the catecholaminergic system in the thermoregulatory centre may 'overrule' this safety brake. These results are in line with the results of Watson *et al.* (2005) who examined the effects of bupropion both in temperate and in warm environmental conditions in well-trained cyclists. The major finding of their study was that subjects completed the time trials 9% faster in the heat after bupropion supplementation. Seven out of nine subjects reached T_{core} above 40°C, without any change in the subjective ratings of fatigue or thermal discomfort, implying that bupropion may dampen or override hyperthermia-induced inhibitory signals arising from the CNS to stop exercising, thus potentially increasing the risk of developing heat illness. These results from our previous human study (Watson *et al.* 2005) and the present investigation in rats indicate that the development of central fatigue during exercise is counteracted by

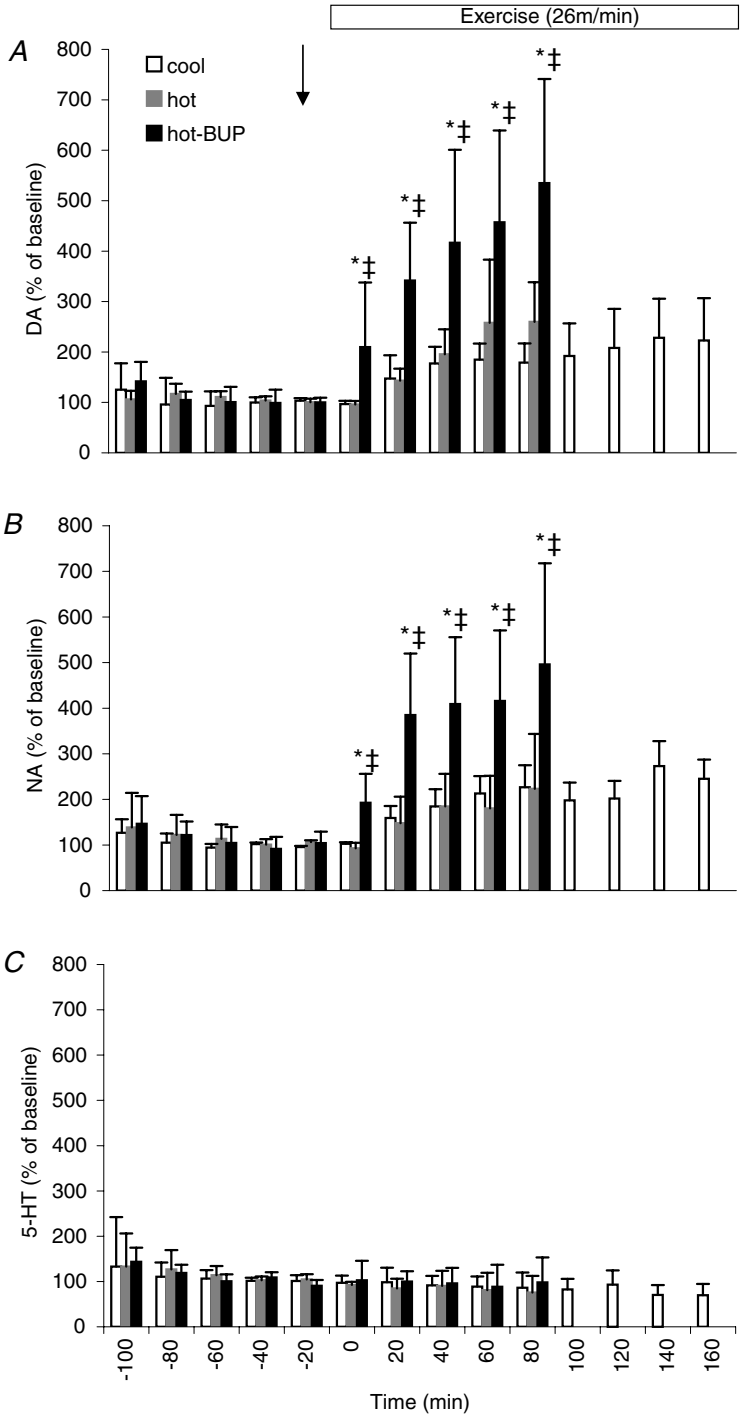

Figure 4. Changes in extracellular dopamine (*A*), noradrenaline (*B*) and serotonin (*C*) levels in the preoptic area and anterior hypothalamus (PO/AH)
The average concentration of three microdialysis samples before treadmill exercise was taken as the baseline and was defined as 100%. Microdialysis samples were expressed relative to the baseline value (mean ± S.D.). *Significant difference, cool *versus* hot-BUP ($P < 0.05$); ‡significant difference, hot *versus* hot-BUP ($P < 0.05$). Values are mean ± S.D. Arrow indicates injection of bupropion or saline.

increasing the DA and NA levels in the PO/AH, which is of major importance for thermoregulation.

It is interesting that T_{brain} was lower than T_{core} at exhaustion (hot: T_{brain}, $40.5 \pm 0.7^{\circ}$C; T_{core}, $41.0 \pm 0.7^{\circ}$C; hot-BUP: T_{brain}, $41.6 \pm 0.7^{\circ}$C; T_{core}, $42.3 \pm 0.5^{\circ}$C). Although it has been reported in rats that arterial blood temperature is lower than the temperature of some brain structures, such as the striatum and cerebellum, under various environmental challenges (Kiyatkin *et al.* 2002), other studies have reported a lower T_{brain}, as we observed (Zhu *et al.* 2006; Kiyatkin, 2007). These groups showed that deep brain temperature in rats under controlled experimental conditions is slightly (0.2–1°C) lower than the deep body temperature. T_{brain} is a physiological parameter that is determined primarily by neural metabolism, and regulated by cerebral blood flow which serves as a body-coupled heat exchanger system penetrating all brain structures. To maintain temperature homeostasis, thermogenic activity of the brain needs to be balanced by heat dissipation from the brain to the body. Heat dissipation from the brain is highly dependent on convective heat removal by the blood, indicating that cerebral blood flow can be considered as a process that is intended to 'cool' the brain (Nybo & Secher, 2004). These results suggest that this safety mechanism prevents a high level of heat storage and excessive hyperthermia, thus protecting the brain from thermal damage. Because all brain cells are affected by high temperature, destruction of endothelial cells of the brain and leakage of serum proteins across the brain–blood barrier are important factors in determining brain oedema (Sharma & Hoopes, 2003), which is the most dangerous acute complication of pathological brain hyperthermia (Sharma & Ali, 2006).

We also investigated exercise performance and thermal response during treadmill exercise of the rat in a cool environment (18°C). Running time to exhaustion in the cool condition (cool, 143.6 ± 21 min) was two times longer in a warm environment (hot, 65.8 ± 13 min; hot-BUP, 86.3 ± 7.2 min; Fig. 1). Both T_{core} and T_{brain} at the point of exhaustion were significantly lower in the cool condition compared to the warm environment (Fig. 2*A* and *B*). Thus, T_{core} at the point of exhaustion in the cool condition did not reach the critical level of internal body temperature (Fuller *et al.* 1998). Fatigue during prolonged exercise in temperate conditions is typically associated with the depletion of muscle glycogen, accumulation of metabolites, inadequate oxygen delivery and the development of hypohydration (Hargreaves & Febbraio, 1998). Therefore, these results suggest that the factors contributing to fatigue during prolonged exercise are not the same in cool and warm environmental conditions and emphasize that thermoregulation, which is a robust homeostatic physiological phenomenon, can be disturbed by manipulating catecholaminergic neural activity.

In exercising rats, tail skin vasodilatation is an essential route of heat loss from the body, because rodents do not dissipate heat though the evaporation of sweat (Shellock & Rubin, 1984). In the present study, T_{tail} had already increased after 10 min of exercise, and maintained high values during exercise. These results indicate that vasodilatation had occurred with a consequent activation of heat loss responses during exercise in the heat. Although the heat dissipation mechanism was present, it seemed insufficient to overcome the intense heat production during exercise in the heat because T_{core} did not reach a plateau or steady level in either hot or hot-BUP groups. We previously showed that an acute injection of bupropion suppressed heat loss responses and elevated T_{core} and T_{brain} in resting freely moving rats in a temperate environment (Hasegawa *et al.* 2005). In the present study, the T_{tail} values recorded at the end of exercise were not significantly different between the bupropion and hot groups, although there was a tendency for T_{tail} of the bupropion group to be lower when compared to the hot group (time, 20 min; $P = 0.10$, Fig. 3). These results suggest that bupropion did not affect heat loss mechanisms during exercise in the heat, but it may alter heat production mechanisms.

Bupropion is a weak monoamine reuptake inhibitor that shows 2.5-fold selectivity for DA *versus* NA, and has no effect on 5-HT (Hyttel, 1982; Richelson & Pfenning, 1984). Previous microdialysis studies in rats have shown that acute administration of bupropion affects DA release in the striatum and nucleus accumbens in a dose-dependent manner (Nomikos *et al.* 1989), and affects hippocampal DA and NA release (Piacentini *et al.* 2003). We have also confirmed that an acute injection of bupropion increased the extracellular concentrations of DA and NA, but not of 5-HT in the PO/AH in freely moving rats (Hasegawa *et al.* 2005). In the present study, extracellular concentrations of both DA and NA in the PO/AH significantly increased during exercise following an acute injection of bupropion, was higher in the hot-BUP than in cool and hot groups (Fig. 4*A* and *B*). These results suggest that the combination of exercise and heat with bupropion supplementation influences the catecholaminergic neural activity in the hypothalamus. It is well known that cathecholamines are neurotransmitters linked to the central component of fatigue via their well-known role in motivation and motor behaviour (Chaouloff, 1989; Meeusen & De Meirleir, 1995), and is therefore thought to have an enhancing effect on performance. Bridge *et al.* (2003) indicated that subjects with a high dopaminergic neural activity may demonstrate a higher tolerance to exercise in the heat. In addition, it has been reported that intracranial self-stimulation of the ventral tegmental area, the origin of the dopaminergic projection, maintained a predetermined running speed in rodents (Burgess *et al.* 1991). Therefore, the present results suggest that elevated T_{core} and T_{brain}, due to the high rate of heat production during prolonged

exercise, is accompanied by an increase in DA and NA levels in the PO/AH. In particular, DA through its important role in the mesolimbic reward system seems to be important in overruling the inhibitory signals arising from the CNS, and plays a role in the increase in performance (Tella *et al.* 1996), as found in the present animal study and in our previous experiments (Watson *et al.* 2005). The physiological mechanisms involved in hyperthermia-induced central fatigue may be influenced by neurotransmitter activity of the dopaminergic system.

Another interesting finding of our study is a linear correlation between final T_{brain} and T_{core} and neurotransmitter levels in the PO/AH at exhaustion. Statistical analysis of final T_{brain} and T_{core} and neurotransmitter levels at exhaustion showed a good correlation only with changes in DA and NA but not with 5-HT. Thus, DA and NA neurotransmission might be involved in exercise performance and thermoregulation in the heat; however, future studies need to elucidate which neurotransmitter is involved in these functions.

Conclusion

The data from the present study indicate that an acute injection of bupropion in rats enhances exercise performance, T_{core} and T_{brain} during exercise in a warm environment with an increase in the extracellular concentrations of DA and NA in the thermoregulatory centre. These results support previous findings in humans that showed an increased performance and T_{core} with acute bupropion supplementation in the heat. Moreover, another important finding of the present study was the increase in T_{brain} and specific neurotransmitters in the thermoregulatory centre during exercise in the heat. These findings suggest that this drug could be employed as a potential performance-enhancing agent when competing at high ambient temperatures. Together, the present and previous results (Watson *et al.* 2005; Hasegawa *et al.* 2005) investigating acute bupropion supplementation during exercise in the heat should be taken into consideration by the sports medical world, including the World Anti-Doping Agency (WADA) and specifically the International Olympic Committee, which removed this drug from the list of prohibited substances in January 2003. This should be considered not only because the drug improves performance, but also because of the potential risks athletes might encounter if bupropion is taken acutely while exercising at elevated temperatures. In addition, bupropion is still part of the monitoring programme of the prohibited list of WADA 2007.

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